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THE EFFECT OF THE INTERACTION BETWEEN TWO MUTATIONS MSTN/T434C AND CAST/T350C ON CARCASS AND MEAT TRAITS IN ARABI SHEEP IN IRAQ

Abdullah Hameed SALIM[1]

University of Thi-Qar, Iraq

Abstract

This study was carried out on a sample of 49 Iraqi Arabi sheep in a slaughterhouse in the Thi Qar Governorate in southern Iraq . Laboratory tests were done in the physiology lab of the Faculty of Agriculture and the Marshes at University of Thi Qar .The study aimed to find out the impact of the interaction between two mutations (MSTN/T434C and CAST/T350C) on the Myostatin and Calpastatin genes, respectively, on some characteristics of sheep carcass and meat after slaughtering sheep and recording the sex and age of the animal. The results of the study showed a significant difference (P≤0.05) of the interaction between the two mutations in the characteristics of live body weight, dressing ratio and carcass diameter by the superiority of the CCTT genotype in the characteristic of live body weight and the superiority of the TTTC genotype in the characteristic of the dressing ratio, while the superiority of the TTCC genotype in the characteristic of the carcass diameter and neither of the two traits showed both the hot weight and length of the carcass significant differences between the four genotypes that appeared between the resulting interactions. Significant differences (P≤0.05) appeared for the effect of the interaction between the two studied mutations on the Myostatin and Calpastatin genes in the meat pH characteristics and the muscle fibers breakage index, where the TCTC interaction outweighed the TTCC interaction while it did not differ significantly with the other three interactions in the pH. As for muscle fiber fracture index, the TTCC genotype was significantly superior to the CCTT genotype, but it was not significantly superior to the TTTC and TCTC interference, respectively.

Keywords: Arabi Sheep, Carcass Traits, Mutations Interaction, CAST And MSTN Genes.

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[1] abdallah@utq.edu.iq

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Introduction

Sheep are one of the important agricultural animals in Iraq, with a number of 9.350 million heads with goats (FAO, 2010). Sheep in Iraq consist of Karady, Arabi, Awassi and Nuaimi breeds, all of which are classified within the family of wool sheep with a fatty tail, which is considered one of the most types of sheep that tolerate difficult environmental conditions and resistance to diseases compared to breeds others lack the fatty tail (Majid et al., 2003). Arabi sheep are found in most of the southern provinces of Iraq, and they constitute about one-fifth of the total Iraqi sheep which consider the smallest in size and the most resistant to difficult environmental conditions. Its wool is characterized by being one of the best Iraqi wools of white, brown, black and red colours, as the weight of the fleece reaches 1,250 kg (Jayed, 2008). In general, Iraqi sheep are bred primarily for the purpose of producing meat, milk and wool products (Jumah. and Alkass, 2000). sheep in general contribute about 6% of the total red meat produced in Iraq (El Fiky et al., 2017), Therefore, unremitting efforts are being made for the purpose of increasing production levels in livestock, especially sheep, and many different means have been adopted for this purpose. The use of biotechnologies as a means of early selection in animals of economic importance among several modern methods, which gave important results in the development of the reality of livestock at the global level. The Iraqi domestic breeds shortage genetic diversity and will therefore cofront difficulty in modification to changing diseases and environmental factors (Khodabakhshzadeh et al., 2016). Therefore, the sites of genetic excellence for local breeds must be studied to preserve their genetic diversity, as well as knowing the most important performance characteristics that can be predicted at their levels based on a study animals genetically (Shojaei et al., 2010). Rodgers and Garikipat(2008) indicated that within the main genes that have confirmed their relationship and association with many frugal characteristics in different animal species is the Myostatin (*MSTN)* and Calpastatin (*CAST*) genes, where the first is called as the growth and differentiation factor 8 (GDF8) which located in sheep (Ovis aries) on chromosome number 2 and is consist of three exons and two introns. Embryogenesis is the main process which Myostatin gene is involved, also in tissue homeostasis in all adult animals and appear to be a negative regulator of growth and muscle development, and is expressed in both skeletal muscle in growth and adulthood and through various research shows that deletions or mutations in *MSTN* are responsible for the phenotype of double muscle (McPherron et al., 1997). Several studies indicated that *MSTN* gene polymorphisms have many impacts on different characteristics such as reproductive efficiency, animal health, body weight, dressing ratio and weight gain (Paswan et al., 2014). The second gene (Calpastatin) is located on the fifth chromosome in sheep (Yilmaz et al., 2014). Calpastatin activity is closely related to muscle growth rate, meat tenderness and meat quality characteristics through its role in inhibiting Calpain activity and thus reducing the rate of protein degradation (Greguła -Kania 2012). Increasing the production of calpastatin enzyme increases muscle growth and distribution to the skeleton in animals during birth and this effect continues until weaning (Byun et al. 2008), as well as many studies indicated that there is a link between the polymorphisms of *CAST* gene with different growth traits in sheep and goats (Dehnavi et al, 2012) and with the characteristics of the carcass (Schenkel et al, 2006). The myostatin and Calpastatin genes, are often expressed in skeletal muscles, they have the role of controlling the pervasion of muscle cells and the figuration of muscle fibers (Cieslak and colleagues. 2003). As a hormonal negative regulator (Du et al., 2007) a mutation in myostatin gene can lead to a decrease in expression as in the excluded animals due to a threefold increase in their muscle mass due to muscle fiber hypertrophy or overexpression that leads to muscle cell hypertrophy (Lee and McPheron, 2001). In addition to identifying genetic polymorphisms, determining the levels of active gene expression in muscles can also help us understand the overlap and interactions with each other, and the researchers can analyze the interaction between the mutations and variations on Myostatin and Calpastatin and use them as an aid

in the genetic improvement programs for economic animals. Therefore, the aim of this study was to study one of these interactions between the two genes and relate it to some characteristics of carcass and meat in local Iraqi sheep.

Materials and methods

This study was done in south of Thi Qar Governorate, in Suq Al-Shuyoukh district and included slaughtering 49 Arabi sheep (14 males and 35 females) private slaughter shop taked from one herd and studying main traits of the carcass and then taking a sample of sheep thigh meat to calculating some of the chemical characteristics in the laboratory which belonged to faculaty of Agriculture and Marshes / University of Thi-Qar immediately after taking them, as well as taking blood samples to laboratory of the Marshes Research Center in the University of Thi-Qar to separating the total DNA from animals blood samples and making a molecular examination of the target segment of the Myostatin and Calpastatin genes. The hot carcass weight and dressing ratio were measured after the slaughter process, as well as calculating the length and the diameter of the carcass of the carcass. The pH was measured according to the method of Attken et al. (1967) and the water carrying capacity trait was estimated as in Denhertog method (1997) and diameter of muscle fibers trait was estimated according to the method of Jeremiah and Martin (1977), and the method of Davis et al. (1980) was used to measuring muscle fiber fracture index trait.

The process of DNA extraction from sheep blood samples according to the kit (Bioneer company) instructions. Electrophoresis was used (to ensure the hit of the DNA extraction process) at 70 V. and 85 mA with 30 min.,after that, the agarose gel was examined by a UV Gel Documentation device, and the photos of migration were taken by the installed camera. Primers (Table 1) were selected to knowing the multiplicity of genes and mutations present in the genes.

Table 1: Primers used in the study

Genes	Primer sequencing	Product size	Refrences
Myostatin (*MSTN*)	**F/ 5'- TGCGGTAGGAGAGTGTTTGG - 3'** **R/ 5'- AAAATTGTTGAGGGGAAGACC-3'**	487 bp	Haren et al,2019
Calpastatin (*CAST*)	**F/5'-TGGGGCCCAATGACGCCATCGATG- 3'** **R/5'-GGTGGAGCAGCACTTCTGATCACC- 3'**	622 bp	Jwasreh et al,2016

The primers of the *MSTN* gene were made ready by the Macrogene Company, and the agarose gel was made ready with the same steps that used in total DNA of the samples, except the concentration of the agarose (1.5%) and according to the optimal conditions for the reaction of the *MSTN* and *CAST* genes (Table 2). After confirming the size of the PCR segment for the studied genes by comparing it with the standard DNA Ladder,then, 20 ml. were taken of the PCR product and sent to the Macrogene Company, where the samples were purified and then the sequencing analysis process using the technique of sanger sequencing. The sequencing results were received and analyzed by BLAST on the NCBI World Gene Bank website .

Table 2: PCR amplification reactions

Genes	Stages	Temperature (C^0)	Time (minute)	Cycles
Myostatin and Calpastatin	Initial denaturation	95	5	1
	Denaturation	95	30 second	35
	Annealing *(MSTN)*	58	45 second	35
	Annealing *(CAST)*	62		
	Elongation	72	45 second	35
	Final extension	72	10	1

The data of the study were analyzed by the statistical program (SAS) (2012), using complete random design (CRD) to study the impacts of each of the polymorphisms of the two mutations in *MSTN* and *CAST* genes respectively in the studied characteristics of Iraqi domestic sheep, and using Duncan's polynomial test (Duncan, 1955) to compared between averages.

Mathematical model as follow:

$$Yij = \mu + G_i + E_{ij}$$

Whereas:

Yij: the observation value (j)of genotype (i).

μ: the general range of the traits.

Gi: polymorphism impact of the interaction between Myostatin and Calpastatin genes.

Eij: the random error that is normally distributed with a mean of zero and a variance of δ^2 0.

Results and discussion

In table (3) it is appear the averages of the studied characteristics , which were close to the known general averages of these traits in most of the previous studies.

Table 3: Means and standard error (S.E.) for studied characteristics

Variables	Mean ± S.E.
Live body weight (kg)	37.04±2.25
Dressing ratio (%)	44.90±1.455
Hot carcass weight (kg)	16.90±0.966
Carcass length (cm)	82.28±2.65
Carcass circumference(cm)	79.79±3.77
Meat pH	5.90±0.76
WHC	133.67±3.33
Fiber diameter	0.33±0.02
MFI	1.91±0.11

WHC: water holding capacity, MFI: myofibril fragmentation index.

Table 4 shows the effect of the interaction between the two mutations *MSTN/T434C* and *CAST/T350C* in the study samples on the Iraqi sheep (Arabi) for the characteristics of live weight before slaughter and the characteristics of carcass. Where it is clear that there is a significant difference (P≤0.05) within the four interactions between the genotypes of the two mutations on the myostatin and calpastatin genes obtained from this study for the characteristics of live body weight, dressing ratio and carcass diameter, where the overlap was superior to *CCTT* at a rate of 38.87 kg (which is the highest mean for the trait) in the trait. The live body weight on the *TTTC* interaction, which was 33.44 kg (which is the lowest average for the same trait). As for the dressing ratio, it was reflected in the moral superiority, so that the superiority was for the *TTTC* genotype, with an average of 49.66, compared to the lowest dressing ratio, which was in the *CCTT* genotyp, which amounted to 43.75. The *TTCC* genotype outperformed the interaction between the two studied genes in the carcass diameter characteristic to give the highest values, which reached 85.92 cm compared to the lowest (75.87 cm), which is the *CCTT* genotype.We did not notice any significant difference between the studied interactions for the characteristics of the hot weight and length of sheep carcass.

Table 4: Effect of interaction between MSTN/T434C and CAST/T350C mutations on live body weight and some carcass traits.

Mutation interactions	Number	Variables				
		Live body weight(kg)	Dressing ratio (%)	Hot carcass weight(kg)	Carcass length(cm)	Carcass circumference (cm)
TTTC	9	33.44±1.93 b	49.66±1.74 a	16.50±0.58	80.33±1.67	78.22±2.90 ab
TTCC	14	36.07±2.66 ab	44.03±1.71 b	16.16±1.38	84.42±3.89	85.92±4.41 a
TCTC	9	38.50±2.49 a	44.00±1.42 b	17.30±1.49	84.11±3.27	78.90±4.72 ab
CCTT	17	38.87±1.97 a	43.75±1.15 b	17.56±0.92	80.58±2.29	75.87±2.82 b
Significance	49	*	*	NS	NS	*

TTTC: TT *MSTN* with TC *CAST*, TTCC: TT *MSTN* with CC *CAST*, TCTC: TC *MSTN* with TC *CAST*, CCTT: CC *MSTN* with TT *CAST*. *: (P≤0.05) , NS: non-significant.

In table 5, significant differences (P≤0.05) appeared for the effect of the interaction between the two studied mutations in the pH and myofibrillar defragmentation index (MFI), where the *TCTC* interaction with an average of 6.03 outperformed the *TTCC* interaction (5.73) while it did not differ significantly with the three interactions in the adjective pH. As for muscle

MFI, *TTCC* (2.09) was significantly superior to *CCTT* (1.71), but it was not significantly superior to *TTTC* and *TCTC*, respectively.

Table 5: Effect of interaction between MSTN/T434C and CAST/T350C mutations on meat chemical traits

Mutation interactions	Number	Variables			
		PH	WHC	Fiber diameter	MFI
TTTC	9	5.87±0.06 ab	128.88±2.60	0.32±0.03	1.90±0.16 a
TTCC	14	5.73±0.10 b	138.57±4.55	0.33±0.01	2.09±0.14 a
TCTC	9	6.03±0.04 a	131.11±3.51	0.32±0.02	2.03±0.11a
CCTT	17	6.01±0.08 a	133.52±4.10	0.36±0.02	1.71±0.02 b
Significance	49	*	NS	NS	*

TTTC: TT *MSTN* with TC *CAST*, TTCC: TT *MSTN* with CC *CAST*, TCTC: TC *MSTN* with TC *CAST*, CCTT: CC *MSTN* with TT *CAST*. *: (P≤0.05) , NS: non-significant.

There were no significant differences between the four interactions in both of WHC and fiber diameter traits, despite the simple arithmetic superiority of *TTCC* genotype for WHC trait and *CCTT* for fiber diameter trait (138.57 &0.36 respectively).

Through the above results, it can be concluded that the interaction between the Myostatin and Calpastatin genes was significant in most of the studied traits, which indicates the possibility of relying on it in selection programs and future genetic improvement of carcass traits, as these genes are considered among the main genes affecting meat quality characteristics such as tenderness and muscle fat, as well as the importance of intensive study of the CAST gene is highlighted because it is assume a chosen gene and is attached with the characteristics of body weight and carcass (Hopkins, 2011). At the same time, the MSTN gene is in charge of affecting the phenotypes of meat tenderness, as well as being a gene that controls the proliferation of muscle cells and muscle fibers, and its excessive secretion leads to muscle hypertrophy, which is very related to the expression of the calpastatin gene, and the rate of correlation between the two genes was 0.79 in a study of the expression of the two genes in a number of genetic groups in Brazil (Bagatoli et al. 2013).

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